According to these results, the optimum SPME experimental procedures to extract chlorophenols in urine were with a polyacrylate-coated fibre at pH 1, extraction time 50 min and desorption into the GC injector at 290°C for 2 min. The technique offers a low ng L^{-1} sensitivity to determine trace amounts of chlorophenols in a urine sample containing high levels of interference. The method has been successfully used to analyse urine samples of workers in a sawmill where chlorophenol-containing anti-stain agents were previously used. Analysis indicated that chlorophenols were found in 9 out of 10 urine samples. The concentration of chlorophenols ranged from 0.02 μg L^{-1} (PCP) to 1.50 μg L^{-1} (2,4-DCP).

Future Prospects

Phenols, particularly chlorophenols, are toxic at concentrations of a few μg L^{-1} and are also persistent. Determining trace amounts of phenols is not easy for real samples that consist of extremely complex matrices. Methods for monitoring trace amount of phenols in real samples must be sensitive and selective, and should be rapid and simple. A mixture can be separated by GC into its individual components and, at the same time, the amount of each compound present can be determined. Furthermore, analysis can be performed with various detectors at a moderate cost. The applications presented here demonstrate the effectiveness of GC to analyse quantitatively trace levels of phenols in complex mixtures. Although various GC methods for phenol analysis have been widely used, novel techniques and sample pretreatment methods are continually being introduced. Simple retention times are not very reproducible and linking with a mass spectrometer as a detector is desirable for unambiguous identification. This approach has the merits of speed, sensitivity and selectivity. The MS-MS technique will become less expensive in the future and, eventually, the preferred means of analysing phenols when coupled to GC. Currently GC-MS-MS is more expensive than GC-MS but offers an extra separation stage to resolve the problem of analysing mixtures not amenable to GC-MS.

See also: **II/Chromatography: Gas:** Column Technology; Derivatization; Detectors: Mass Spectrometry; Detectors: Selective; **III/Phenols:** Liquid Chromatography; Solid-Phase Extraction; Thin-Layer (Planar) Chromatography.

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Liquid Chromatography

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Phenols include a considerable range of substances which possess an aromatic ring with one or more hydroxyl substituents. These compounds are present in many different types of sample, which means that the determination of phenolic compounds is of wide interest.

These compounds may be natural or synthetic. They are present in all plant tissues and are frequently the most abundant secondary metabolites in fruits, in which they sometimes reach high concentrations. Phenolic compounds may also be found in combinations to form flavones and glucosides in trees and plants.

Apart from their natural origin, phenols are also breakdown products from natural compounds such as lignins, tannins and humic substances. Chlorinated nitrophenols are the main degradation products of many chlorinated phenoxy acid herbicides and organophosphorus pesticides.

Phenolic compounds are extensively used in diverse products, such as plastics, dyes, antioxidants, cosmetics, pharmaceuticals and paper.

Some phenolic compounds are claimed to have medicinal properties, and are used in ointments and creams because of their antifungal, disinfectant and anaesthetic properties. Some compounds, namely caffeic, chlorogenic, ferulic, gallic and ellargic acids, have been found to be pharmacologically active as antioxidant, antimutagenic and anticarcinogenic agents.

As a result of emission accidents and other releases, phenolics are present in the environment and chlorophenols particularly constitute an environmental problem owing to their possible presence in rivers, lakes and seas where they may enter the food chain. Some phenols are toxic to humans and aquatic organisms and can cause serious taste and odour contamination even at very low levels.

Thus, the types of sample in which phenolic compounds are to be determined are diverse and include biological fluids (serum, whole blood, urine), industrial products and process streams, medicinal creams and ointments, dyes and environmental samples such as air or water (including wastewater, surface water and tap water).

Table 1 shows the main classes of phenolics in fruits and **Table 2** shows the phenolic compounds included on the priority pollutants list of the European Community and the US Environment Protection Agency (EPA).

The determination of individual phenolic compounds requires chromatographic techniques because of the large number of compounds with similar structures. Of these techniques, gas chromatography involves the derivatization of most phenolic compounds, which increases the time of analysis and introduces the possibility of additional errors. Supercritical fluid chromatography does not significantly improve their analysis compared to the other chromatographic techniques and an organic modifier, such as methanol, must be added to CO_2 for the mobile phase. Capillary electrophoresis, by both capillary zone electrophoresis and micellar electrokinetic chromatography, may be used in the determination of phenolic compounds but its limited concentration sensitivity, even when online preconcentration techniques are used, has so far restricted its application.

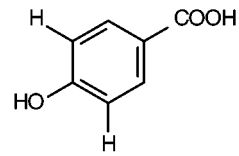
Thus, the recommended technique for determining phenolic compounds is either gas or liquid chromatography, the latter being the most used.

In some kinds of sample, the concentration of phenols may be very low and the detection systems not sensitive enough to detect them, so a preconcentration step is often required. Some samples also require a clean-up step in order to prevent possible interferences from the matrix.

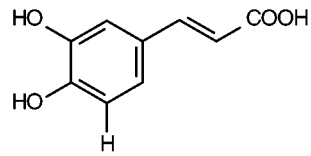
Table 1 Main classes of phenolics in fruits

Number of carbon atoms	*Basic skeleton*	*Class*	*Example*	*Fruit (example)*
7	C_6–C_1	Hydroxybenzoic acids	*p*-Hydroxybenzoic	Srawberry
9	C_6–C_3	Coumarins	Scopolin	Citrus
	–	Hydroxycinnamic acids	Caffeic	Apple
10	C_6–C_4	Naphthoquinones	Juglone	Walnut
13	C_6–C_1–C_6	Xanthones	Magniferin	Mango
14	C_6–C_2–C_6	Stilbenes	Resveratrol	Grape
15	C_6–C_3–C_6	Flavonoids	Cyanidine	Cherry
		Isoflavonoids	Daidzein	French bean
n		Lignins		Stone fruits
		Tannins		Persimmon

Examples



p-Hydroxybenzoic acid



Caffeic acid

Table 2 Phenolic compounds included in priority pollutants list of the EC and US EPA (method 604 and 8041)

Commission of the European Communities (directive 76/464/EC)	
2-Amino-4-chlorophenol	
4-Chloro-3-methylphenol	
2-Chlorophenol	
3-Chlorophenol	
4-Chlorophenol	
Pentachlorophenol	
Trichlorophenols	
US EPA list of priority pollutants (EPA 8041)	
Phenol	4-Chloro-3-methylphenol
2-Methylphenol	3-Methylphenol
4-Methylphenol	2,4-Dimethylphenol
2-Chlorophenol	2,4-Dichlorophenol
2,6-Dichlorophenol	2,4,6-Trichlorophenol
2,4,5-Trichlorophenol	2,3,4,5-Tetrachlorophenol
2,3,4,6-Tetrachlorophenol	2,3,5,6-Tetrachlorophenol
Pentachlorophenol	2-Nitrophenol
4-Nitrophenol	2,4-Dinitrophenol
Dinoseb	2-Cyclohexyl-4,6-dinitrophenol
4,6-Dinitro-2-methylphenol	

Liquid Chromatography Separation

Reversed-phase separation is by far the most efficient technique for separating phenolic compounds. Several kinds of column have been used, although C_{18}-bonded silica seems to be the preferred stationary phase. When 29 phenolic compounds with chloro-, nitro-, hydroxy-, methoxy-, ethoxy-, aldehyde-, and carboxylic functionalities were separated in different columns (a polymer functionalized silica, a polystyrene divinylbenzene polymer, a carbonaceous phase and a silica C_{18}), the best results were obtained with the C_{18} silica. The best overall separation is obtained with the C_{18} column, but a carbon column may be better for the more polar compounds.

When the C_{18}, diphenyl and propylnitrile columns are used with different gradient elutions, the best resolution is also obtained on a C_{18} stationary phase.

It should be pointed out that, depending on the supplier, the characteristics of the column may differ slightly. It is also important to take the dimensions of the columns into account. Several lengths (between 100 and 300 mm) of stainless-steel columns of 4.6 mm i.d. are commonly used, but microbore columns have also been used to reduce solvent consumption, shorten analysis time, increase sensitivity and allow the injection of smaller sample volumes. However, microbore columns are limited because of the higher interference from the matrix components and the changes in flow rates, both of which considerably shorten the life span of the column.

Phenolic compounds can be separated by isocratic or by gradient elution. Gradient elution is generally based on the modification of the organic solvent, mainly methanol or acetonitrile. Gradient elution is usually preferred when phenols covering a wide range of polarity are to be determined. However, it may take longer to stabilize the analytical column and there may be changes in the baseline due to the changes of the mobile phase.

When isocratic elution is used, separation is good for the more polar compounds but dispersion is significant in the late eluted peaks, which means a decrease in sensitivity. So in some cases, such as the determination of phenols of environmental concern, two elutions at different percentages of organic solvent are recommended when isocratic elution is used, due to the different polarity of phenol and pentachlorophenol. For isocratic elution, the instrumentation is simpler since only one pump is necessary. Isocratic separation is preferred when electrochemical detection is used because gradient elution involves a significant decrease in sensitivity. However, for most applications gradient elution is used.

Figure 1 shows a chromatogram of a standard solution of seven phenolic compounds under isocratic elution.

The composition of the mobile phase depends on the type of detector used. In general, the pH of the mobile phase and the percentage of organic solvent are the most important parameters to be optimized.

The pH of the mobile phase is known to influence the retention of phenols on the column depending on their protonation, dissociation or partial dissociation. Partial dissociation might lead to additional peak broadening and asymmetric peaks due to co-elution of the acid solute of the component and its conjugate base. The influence of this effect depends on the K_a values of the compounds. The most common pHs used for the separation of phenolic compounds are between 2.5 and 3, where the analytes are separated in their acidic form, and 5–7, when some of the phenolic compounds are in their dissociated form. Depending on the phenolic compounds to be separated, one pH value or another may give better separations. It is also usually recommended to work with buffer solutions to adjust the pH of the mobile phase. Acetic acid is the most used acid to adjust the pH, although sulfuric acid is also used, and buffer solutions of phosphate are the most used. **Table 3** summarizes some pH and buffer solutions, analytical columns and detection conditions used for the determination of phenolic compounds in different samples.

Some phenolic compounds can also be determined by ion interaction reversed-phase HPLC. The method is based on the ability of phenolic compounds to form ion pairs with alkylammonium ions and, for instance,

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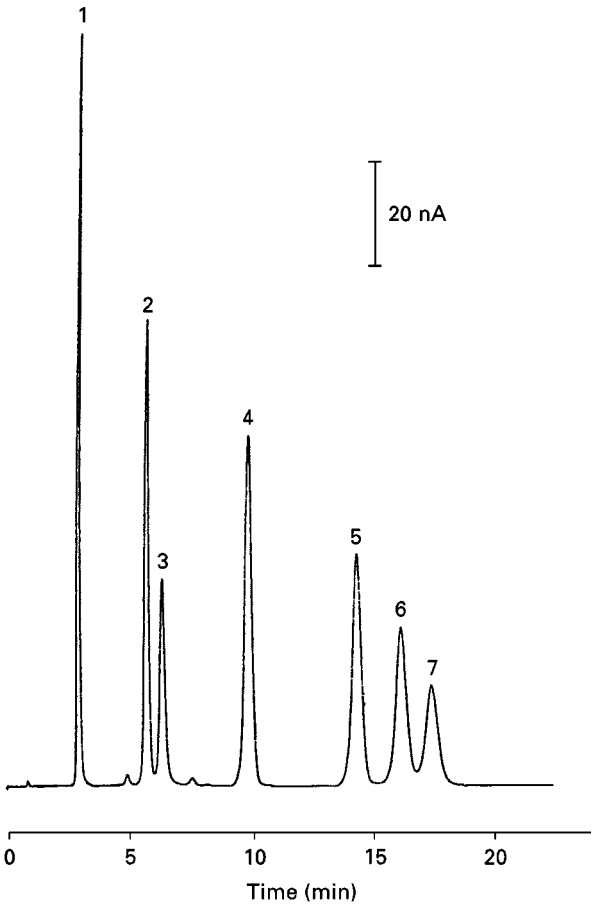

Figure 1 Chromatogram of a standard solution (20 ng for each compound). Analytical column: 125 × 4 mm i.d. LiChrospher 100 RP-18. Mobile phase: 30 mmol L^{-1} sodium monohydrogen–phosphate/sodium dihydrogen phosphate, pH 7.0–acetonitrile–methanol (64 : 19 : 17, v/v/v) at 1.4 mL min^{-1}. Coulometric detection, applied potential 750 mV vs Pd. Peaks: 1, phenol; 2, *o*-chlorophenol; 3, 2,4,6-trichlorophenol; 4, 2,4-dimethylphenol; 5, 4-chloro-3-methylphenol; 6, 2,4-dichlorophenol; 7, pentachlorophenol. (Reprinted with permission from Galcerán MT *et al.* (1995) *Analytica Chimica Acta* 304: 75.)

the 11 EPA priority phenolics may be determined with a mobile phase of water–acetonitrile solution of octylammonium *o*-phosphate at pH 8.

Phenols may be separated by micellar reversed-phase liquid chromatography. For instance, phenolic compounds and their corresponding glucuronides have been determined in urine by isocratic elution using a mobile phase which contains acetonitrile and cetyltrimethylammonium bromide. The use of this micellar agent means that the selectivity of the analytes must be high relative to the urine matrix components and it allows the glucuronides and parent compounds to be simultaneously analysed without the need for gradient elution.

Some compounds may also be added to the mobile phase in order to form complexes with the phenolic compounds and enhance the detection response: for instance, α-cyclodextrin can be added to the mobile phase in order to enhance the fluorescent properties of phenolic compounds.

Detection

The method of detection which is most used in liquid chromatography for the determination of phenolic compounds is UV spectrophotometry, although in recent years electrochemical detection, fluorescence, chemiluminescence and mass spectrometry have been used to increase the sensitivity and selectivity. Their application is described below.

UV Spectroscopy

This is the most widely used technique and each group of phenolic compounds is characterized by one or several UV light absorption maxima. For instance, phenol and chlorophenol derivatives are usually detected at 280 nm, whereas nitrophenols and pentachlorophenol are usually detected at 310 nm. As regards other phenolic compounds, 220 and 275 nm are characteristic of flavanols and hydrocalchones, while 260 and 350 nm are characteristic of flavonols.

Diode array detectors (DAD) are recommended because spectral libraries can be used for confirmation purposes. In complex matrices, identifying spectra by comparison is extremely useful and DAD enables each peak to be measured at its maximum wavelength of absorbance, which means an increase in sensitivity. DAD also makes it possible to detect overlapped peaks when their spectra are different enough.

Phenolic compounds may also be derivatized pre- or post-column in order to enhance their absorptivity in the UV-visible region but derivatization is not commonly used because of the increase in complexity of the method.

Electrochemical Detection

Electrochemical detection (EC) is more sensitive than UV detection for such phenols as phenol and chlorophenols and common breakdown products from lignin such as vanillin, syringealdehyde and *p*-coumaric acid. However, sensitivity does not increase significantly for nitrophenols. The electrochemical conditions depend on the oxidation and/or reduction potential of the solute. The operational potential in most cases is a compromise between the optimal faradaic current and the lowest level of background current for each solute. The electrochemical oxidation of phenolics requires the use of high applied potentials – around 1 V versus a standard calomel

Table 3 High performance liquid chromatography conditions for the determination of phenolic compounds

Compounds	*Column*	*Mobile phase*	*Detector*	*Comments*	*Reference*
18 EPA priority phenolics	Waters C-18 150 × 3.9 mm i.d.	Gradient: A 1% acetic acid B MeOH acetonitrile (1/3)	UV: 310 nm PCP and mononitrophenols 280 nm rest of compounds	TSP for confirmation Natural waters off-SPE	Puig and Barceló (1995)
11 Phenolic compounds	Spherisorb ODS-2 250 × 4 mm i.d.	Gradient: A 1% acetic acid with 0.05 g L^{-1} KCl B MeOH	UV: 316 nm 4-NP 280 nm rest of compounds EC: 1 V	Environmental waters online SPE	Pocurull *et al.* (1996)
18 EPA priority phenolics	Hypersyl green ENV C_{18} 150 × 4.6 mm i.d.	Gradient: A 1% acetic acid B MeOH acetonitrile (1 : 1) 1% acetic acid	APcI 30 V cone voltage	Post-column addition MeOH containing 0.1 mol L^{-1} TEA	Puig *et al.* (1997)
13 Phenolic compounds	Hypersil C_{18} 125 × 4 mm i.d.	Isocratic 30 mmol L^{-1} acetate/acetic acid (pH = 5.3)–acetonitrile–MeOH (60 : 30 : 10)	EC (dual electrode) 750 mV (vs. Pd)	Environmental waters offline SPE	Galcerán and Jáuregui (1995)
Hydroxybenzoic acids Hydroxycinnamic acids	Spherisorb ODS-2 250 × 1.1 mm i.d.	Gradient: A phosphate buffer pH2.4 B MeOH	UV 280 nm hydroxybenzoic acid 320 nm hydroxycinnamic acid	Wine samples SPE	Buiarelli *et al.* (1995)
Hydroxybenzoic acids Hydroxybenzaldehydes	Lichrospher RP-C-18 250 × 4 mm i.d.	Gradient: A MeOH–acetic acid–H_2O (5 : 2 : 93) B MeOH–acetic acid–H_2O (90 : 2 : 8)	DAD 240–390 nm	Brandy samples	Barroso *et al.* (1996)
Hydroxybenzoic acids Hydroxybenzaldehydes	Waters Nova Pak 150 × 3.9 mm i.d.	Isocratic: MeOH–H_2O (0.1% acetic acid, 0.2m mol L^{-1} $(C_2H_5)_4NI$, pH 5.7)	ESP 56 V cone voltage	Fibre samples LLE	Giocchini *et al.* (1996)
Caffeic, chlorogenic, ferulic and gallic acids	Lichrospher 100 RP-18 150 × 3.9 mm i.d.	Isocratic: H_2O–ethyl acetate–acetic acid (95.6 : 4.1 : 0.3)	UV 280, 320, 360 nm	Juices LLE	Shahrzad and Bitsch (1996)

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Table 4 Detection limits (μg L^{-1}) in groundwater using online procedures with UV and electrochemical detection

Compound	*UV*	*EC*
Phenol	10	0.02
4-Methylphenol	1.5	0.01
2,4-Dimethylphenol	0.8	0.03
2-Nitrophenol	1.2	2
4-Nitrophenol	0.8[a]	3
2,4-Nitrophenol	0.5	3
4-Chloro-3-methylphenol	2	0.01
2-Chlorophenol	1.5	0.05
3-Chlorophenol	1.7	0.05
4-Chlorophenol	1.5	0.05
2,4-Dichlorophenol	2	0.03
2,4,6-Trichlorophenol	2	0.03
2,3,5-Trichlorophenol	2	0.03
2,3,4-Trichlorophenol	2	0.03
3,4,5-Trichlorophenol	2	0.05
Pentachlorophenol	1[a]	0.03

Sorbent: PLRP-S; UV detection, 230 nm ([a]310 nm); EC detection, 1 V; sample volume, 10 mL. Reprinted from Puig and Barceló (1995), with permission from Elsevier Science.

electrode which opens up the possibility of fouling the electrodes. Two meta hydroxyl groups in the ring will increase the oxidation potential by some 500 mV. Such a high potential value makes it possible for other matrix compounds to be oxidized, thus increasing the background current.

The increase in sensitivity is very important when isocratic elution is used, although it is lower for gradient elution. Another problem encountered with the use of high applied potentials is the competition between the oxidation of phenols and their electropolymerization which takes place at the electrode surface, thus fouling the surface and giving rise to a decline in signal response with time.

Table 4 compares the limits of detection obtained in groundwater using online solid-phase extraction–liquid chromatography with UV and electrochemical detection. It shows lower limits of detection for most phenols with electrochemical detection, although for nitrophenols, as already mentioned, limits of detection are lower with UV detection.

Pulsed amperometric detection (PAD) is more stable and its greater sensitivity for phenolic compounds makes it highly appropriate for detecting them. Using a working potential which is sufficiently positive to oxidize the phenols electrochemically, the current obtained is proportional to the concentration of the analyte. As the electrochemical conversion results in electrode fouling, a high oxidative potential pulse is applied immediately after the elution of the analytes which strips the fouling products from the electrode. The pulse is followed by another pulse which is lower than the working potential to reduce impurities and clean the electrode surface. This self-cleaning method allows the user to carry out a large number of analyses without cleaning the electrode.

It has been demonstrated that the PAD technique may be used with 200 injections of severely contaminated river water containing di-, tri-, tetra- and pentachlorophenols at a global concentration of about 5 ppm.

The use of chemically modified electrodes (CME) circumvents some of the disadvantages of phenol detection.

A multielectrode electrochemical detector consists of four coulometric array cell modules with four electrochemical detection cells; each may also be used for detecting phenols. The detectors with porous graphite working sensors and palladium reference and counter electrodes are arranged in series after the analytical column. The advantages of this system are that each compound is detected at its highest sensitivity potential and that each peak can be confirmed by comparing the matching ratio R of the standard and the actual sample. R is the ratio between the response from the subdominant channel.

Coulometric array detection is designed so that the eluent flows through a porous graphite electrode having a large cell constant which in turn increases the sensitivity and the signal stability. Unlike common electrochemical detectors in which the electrode typically reacts to 10% or less of the injected sample, coulometric sensors convert 100% of the analyte because phenols oxidize in the high porosity electrode.

Fluorescence and Chemiluminescence

Fluorescence and chemiluminescence detection are known to be very selective and sensitive. Phenols themselves are not fluorescent but they may be converted into a fluorescent compound through a derivatization reaction or by the addition of additives to the mobile phase, in order to make the phenols fluoresce.

The chemical reaction can be in the pre- or the post-column mode. Phenols can be detected by a post-column derivatization procedure, photochemical decomposition of the dansyl derivatives of the phenolic compounds and fluorescence detection. The UV irradiation leads to the formation of highly fluorescent dansyl derivatives which are several orders of magnitude more sensitive than nonirradiated derivatives.

Phenolic compounds can also be determined fluorimetrically by measuring cerium(III), which is the result of oxidizing phenols with cerium(IV) and low limits of detection can be reached.

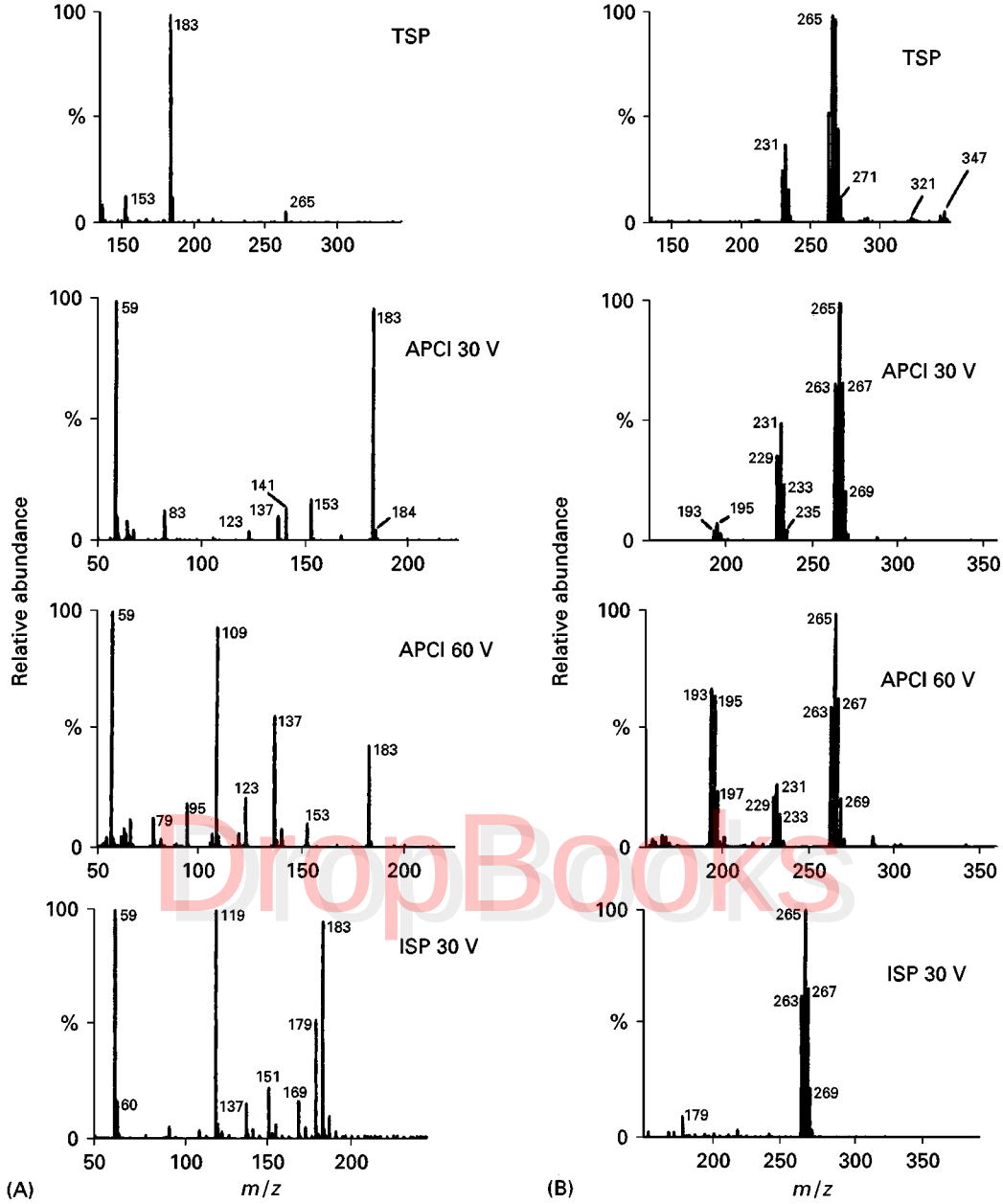

Figure 2 Mass spectra obtained in flow injection analysis using TSP, APCI (30 V cone voltage), APCI (60 V cone voltage) and ISP (30 V cone voltage) for (A) 2,4-dinitrophenol and (B) pentachlorophenol. Conditions: TSP, 1 mL min^{-1} methanol (1 μg mL^{-1} analyte)–water (1% acetic acid) 1 : 1 and post-column addition of 0.2 mL min^{-1} 50 mmoL L^{-1} ammonium acetate; APCI, 1 mL min^{-1} methanol (1 μg mL^{-1} analyte)–water (1% acetic acid) 1 : 1; ISP: 0.3 mL min^{-1} [methanol (1 μg mL^{-1} analyte)–water (1% acetic acid)–methanol (0.1 mol L^{-1} TEA)]. (Reprinted with permission from Puig *et al.* (1996). *Journal of Mass Spectrometry* 31: 1297.)

α-Cyclodextrin can be added to the mobile phase to form inclusion complexes with analytes, such as *p*-hydroxybenzoic, ferulic and vanillic acids and methyl paraben. The inclusion complexes fluoresce allowing detection limits of 1–5 ng L^{-1}.

Chemiluminescence detection may give low detection levels, in the range of 10–100 times lower than for fluorescence detection. The generally preferred detection system is the peroxyoxalate chemiluminescence system. The alkyl-, nitro- and chlorophenols can be detected by using both pre- or post-column derivatization. The method consists of dansylation, photolysis of substituted phenols and peroxyoxalate chemiluminescence detection.

Mass Spectrometry

Mass spectrometry (MS) has become an increasingly attractive technique as a result of the rapid

Table 5 Instrumental detection limits in SIM mode using LC-MS

Compound	*MDLs (ng)*		
	TSP	*APCI*	*ISP*
Catechol	2	0.004	0.740
Phenol	ND	ND	0.175
2-Nitrophenol	1.5	0.050	0.045
4-Nitrophenol	0.4	0.002	0.120
2,4-Dinitrophenol	0.7	0.004	0.256
2-Amino-4-chlorophenol	3.1	0.050	0.500
4-Chloro-3-methylphenol	4.5	ND	3
4-Methylphenol	ND	ND	0.400
2,4-Dimethylphenol	ND	ND	6
2-Chlorophenol	5	0.085	3
3-Chlorophenol	4	0.045	2
4-Chlorophenol	4	0.040	2
2,4-Dichlorophenol	3	0.007	1.310
2,4,6-Trichlorophenol	0.95	0.004	0.330
2,3,5-Trichlorophenol	0.90	0.003	0.350
2,4,5-Trichlorophenol	0.90	0.003	0.300
3,4,5-Trichlorophenol	0.90	0.002	0.300
Pentachlorophenol	0.5	0.001	0.100

MDL, method detection limit; ND, not detected up to 2000 ng. Reprinted from Puig and Barceló (1996), with permission from Elsevier Science.

developments and improvements in interfaces to couple MS to high performance liquid chromatography (HPLC) in recent years. The interfaces which are most used are those based on ionization at atmospheric pressure such as electrospray (ESP), ionspray (ISP) and atmospheric pressure chemical ionization (APCI), although thermospray (TSP) has also been widely used in the recent past.

The TSP interface provides a good response in the negative ion mode for the phenolic compounds on the US EPA list of priority pollutants except for phenol, 4-methylphenol and 2,4 dimethylphenol because they cannot be deprotonated by current buffers even at a high buffer concentration level. The positive ionization (PI) mode shows no signal response for the chlorophenols of environmental interest since the ammonium ions of the mobile phase are not able to protonate them. This is circumvented in the negative ionization (NI) mode where the high intensity of $[M\text{-}H]^-$ is the result of the electron-attracting chlorine group in the aromatic moiety. Other phenolic compounds, such as 4-hydroxycoumarin, 7-hydroxycoumarin and 3,5-dimethoxyphenol, exhibit good responses under the PI mode of operation and some of the main peaks are $[M\text{-}H]^+$ and $[NH_4]^+$.

The main advantages of atmospheric pressure interfaces are the resulting higher sensitivity (especially when using APCI), robustness and ease of use. What is more, by increasing the extraction voltage, structural information can be obtained via collision-induced dissociation using a simple quadrupole instrument.

An ES interface may be used to analyse phenolic compounds. It is compatible with conventional solvent mixtures used for normal or reversed-phase HPLC, up to 80% of which can be water, but only volatile buffers or counterions may be used. The flow rate should be kept low, which is easily done by post-column splitting or by using a semimicro- or micro-column.

Plant phenolic compounds may be determined by HPLC-ES-MS. They are separated in a phenyl column by ion-pairing with tetraethylammonium iodide and are then identified in the negative ion mode, in which only their deprotonated molecules $[M\text{-}H]^-$ are generated.

APCI and ISP interfaces give $[M\text{-}H]^-$ as the main ion with an extraction voltage in the range 20–30 V. It should be pointed out that raising the cone voltage leads to a decrease in sensitivity so this parameter should be carefully optimized to get a good compromise between both factors.

An ISP interface may also be used, but since an acidic pH is normally required for the chromatographic separation of phenolic compounds, a buffer must be added post-column in order to generate ions in solution when performing LC-ISP-MS experiments. This is done by using either KOH or triethylamine. By far the most interesting feature of the ISP interface is the detection of phenol, 4-methylphenol and 2,4-dimethylphenol, although a methanol percentage above 85% is required. This is accomplished by using porous graphitized carbon (PGC) columns where excessive retention allows these analytes to be resolved using 100% methanol as mobile phase.

Figure 2 shows the different mass spectra for 2,4-dinitrophenol and pentachlorophenol obtained in flow injection analysis using TSP, APCI at two cone voltage values and ISP. It can be seen that the compounds are fragmented differently in each case.

Table 5 shows the limits of detection for different interfaces obtained under selected ion monitoring (SIM). It can be seen that APCI is more sensitive than the other interfaces by one or two orders of magnitude.

Sample Handling Techniques

The determination of phenolic compounds usually requires pretreatment of the sample prior to injection into the liquid chromatograph. This pretreatment has two main goals: to clean up the sample, particularly when complex matrices are to be analysed, and to concentrate the phenolic compounds when these are present at low levels in the sample.

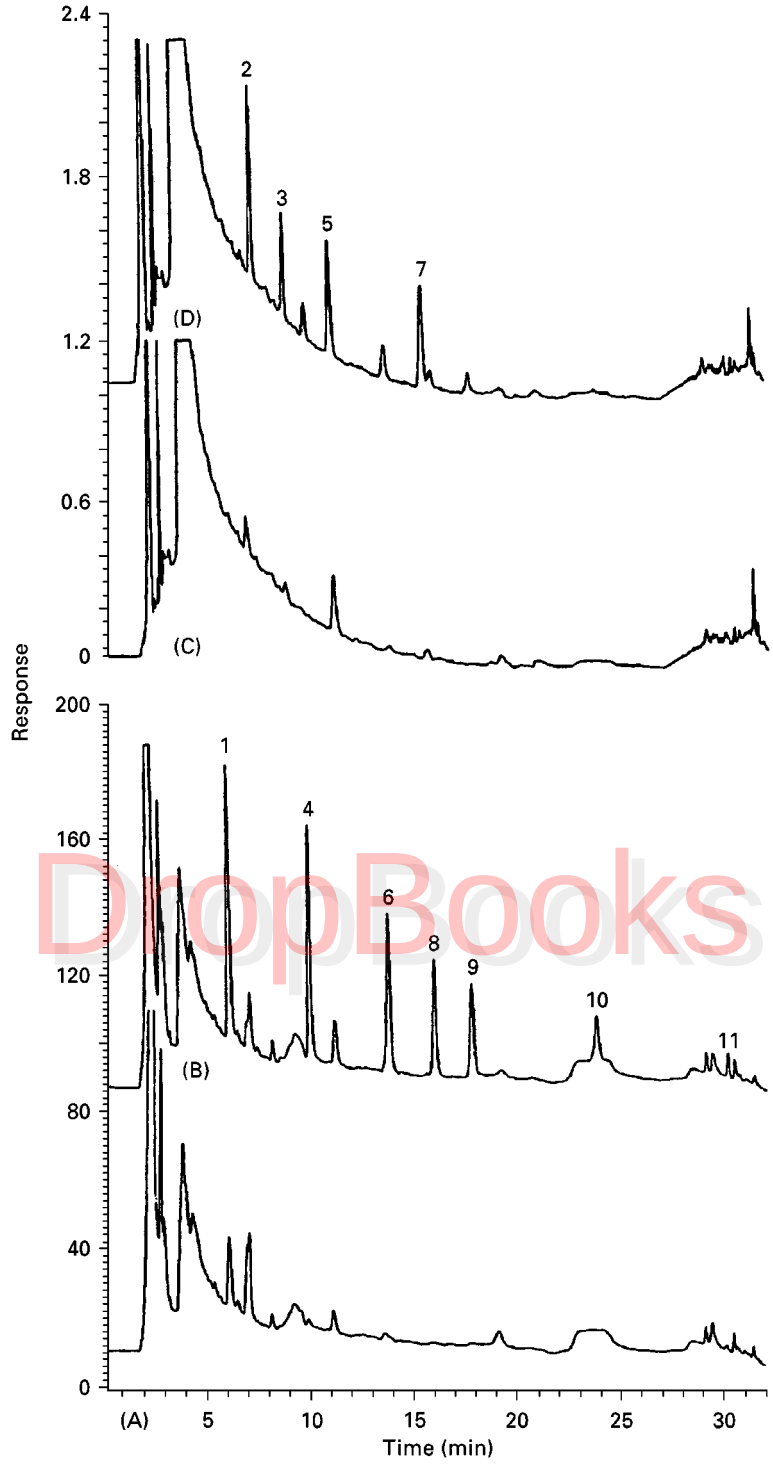

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Figure 3 Chromatograms obtained by online trace enrichment with a PLRP (polystyrene-divinylbenzene sorbent) pre-column and an electrochemical detector (A,B) or a UV detector (C,D) of 10 mL. (A) Ebro river water; (B) Ebro river water spiked at 1 $\mu g\ L^{-1}$ with each phenol; (C) Ebro river water; (D) Ebro river water spiked at 1 $\mu g\ L^{-1}$ with each phenol. 1, Phenol; 2, 4-nitrophenol; 3, 2,4-dinitrophenol; 4, 2-chlorophenol; 5, 2-nitrophenol; 6, 2,4-dimethylphenol; 7, 2-methyl-4,6-dinitrophenol; 8, 4-chloro-3-methylphenol; 9, 2,4-dichlorophenol; 10, 2,4,6-trichlorophenol; 11, pentachlorophenol. (Reprinted from Pocurull *et al.* 1996, with permission from Elsevier Science.)

The easiest way to handle a sample is to combine a concentration with a membrane filtration or ultrafiltration step. This may reduce the level of interfering compounds and eliminates solids and colloids. Liquid–liquid extraction is still a very common sample-handling technique, although it requires a

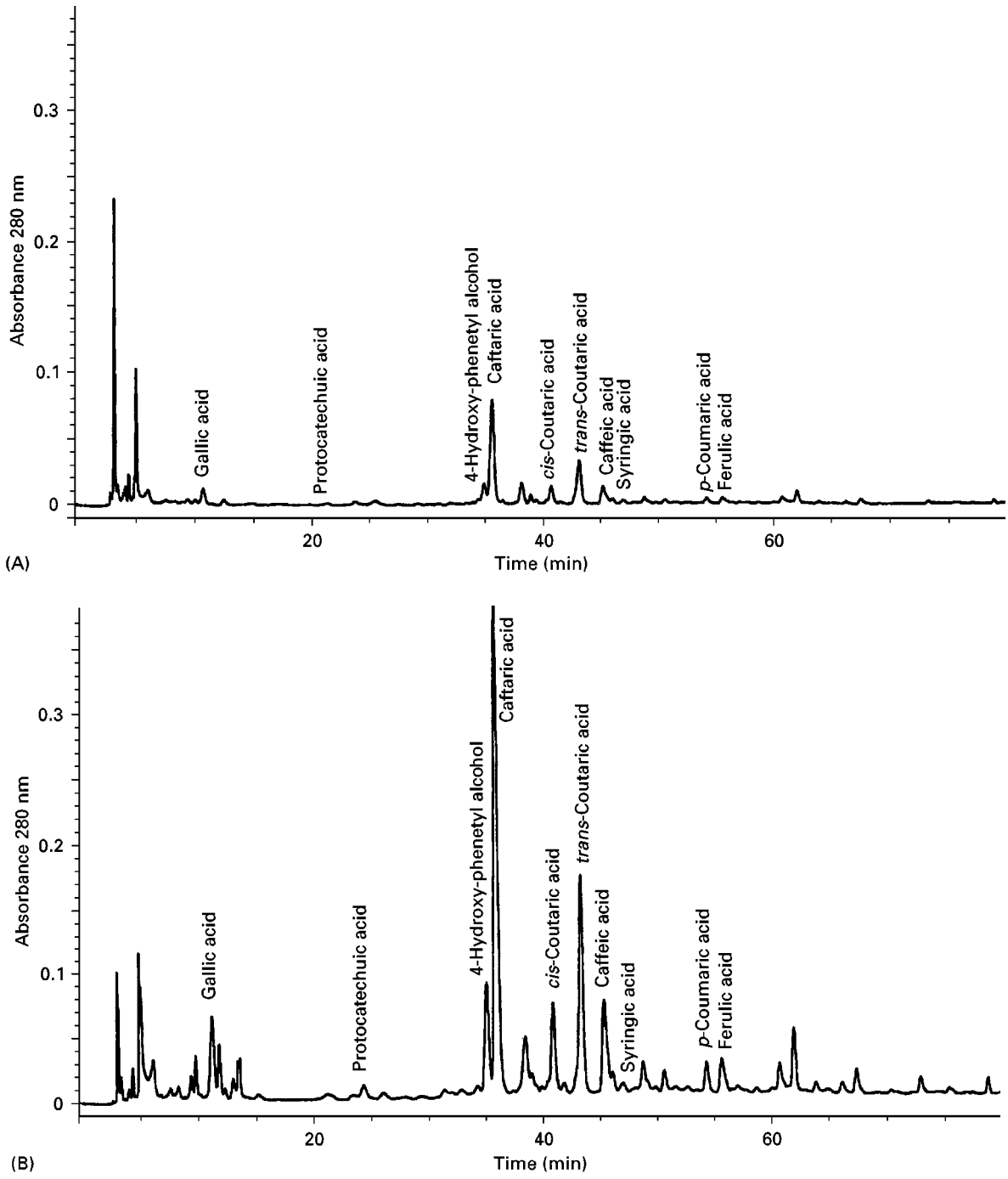

Figure 4 Chromatograms (280 nm) of 20 μL of sherry by direct injection (A) and of 5 mL of sherry after online solid-phase extraction with a polystyrenedivinylbenzene LiChrolut EN (B). (Reprinted from Chilla *et al.* (1996), with permission from Elsevier Science.)

large amount of organic solvents, which is a disadvantage from the environmental point of view.

The most widely used sample clean-up in biological and environmental samples is solid-phase extraction (SPE), which can be used in the offline mode or as an automated online coupling. Online coupling has some very important advantages, such as the automation of the system, lower organic solvent consumption and less handling of the sample. Phenolic compounds can be determined by online SPE reversed-phase LC (SPE-RPLC) with good results as regards limits of detection and precision.

Sorbents such as bonded silica and the polymeric phases can be used in SPE. The low breakthrough volume of most polar phenols in the common sorbents, such as C_{18}-bonded silica and styrene-divinylbenzene,

is considerably increased when highly cross-linked polymer sorbents or chemically modified polymers are used.

The phenolics of environmental concern can be determined at levels required by legislation by using online SPE-RPLC and different sorbents can be used, although the best ones are the highly cross-linked polymeric sorbents.

Figure 3 shows the chromatograms of 10 mL of river water sample obtained by online trace enrichment with a styrene-divinylbenzene (PLRPs) precolumn and HPLC with electrochemical and UV detectors. The figure also shows the chromatograms of the same sample spiked at 1 μg L^{-1} of each phenol.

As regards other kinds of sample, online SPE-RPLC has been successfully applied to the determination of phenolic compounds in beverages, urine and so on.

Figure 4 shows the chromatograms of a sherry, obtained after direct injection of 20 μL (A) and after online SPE of 5 mL of sample (B) and HPLC with UV detection at 280 nm.

In summary, liquid chromatography is a suitable technique for determining phenolic compounds in different kinds of samples. Different detection techniques may be used, some of which, such as EC, enable low levels of phenols to be detected. Mass spectrometry, mainly with APCI, is good for confirming the presence of a phenol in samples at low levels. SPE is the sample-handling technique which is most used for both preconcentration and clean-up of the samples. The combination of this technique, in both the off- and online mode, enables phenols to be detected at low μg L^{-1}.

See also: **II/Chromatography: Liquid:** Derivatization; Detectors: Mass Spectrometry; Detectors: Ultraviolet and Visible Detectors. **Extraction: Solid-Phase Extraction. III / Phenols:** Gas Chromatography; Solid-Phase Extraction.

Further Reading

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Solid-Phase Extraction

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Introduction

Nowadays, sample preparation plays the major role in analysis, as the increasing complexity of samples frequently makes direct analysis impossible. With particular samples, this act is often the limiting factor in the analysis. The aims of sample preparation are as follows: to put the sample in the appropriate physical state for analysis; to clean up the analytes (separate the interference from analytes), and enrichment of analytes. There are many methods of sample preparation which lead to correct analytical results. Features and applications of these methods are presented in numerous compilations and monographs. In this